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Bovine immunodeficiency virus: molecular biology and virus–host interactions

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1. Historical overview

The bovine immunodeficiency virus (BIV) is a lentivirus and one of three morphologically, biologically, and genetically distinct syncytia-inducing retroviruses (Table 1; Fig. 1) discovered in the late 1960s during the intensive search for the cause of enzootic bovine leukemia/lymphosarcoma (Dutcher et al., 1967; Dutcher, 1968; Van Der Maaten et al., 1972; Gonda et al., 1987; Garvey et al., 1990). The other two retroviruses were bovine leukemia virus (BLV), an oncovirus, and bovine syncytial virus (BSV), a spumavirus (Malmquist et al., 1969; Miller et al., 1969). BLV is known now to be the etiologic agent of enzootic bovine leukemia/lymphosarcoma (Burny et al., 1980). BSV has a high prevalence in cattle where it causes persistent infections without apparent clinical disease.

Chronologically, BIV was the third distinct lentivirus discovered and is preceded by equine infectious anemia virus (Vallée and Carré, 1904) and the ovine lentiviruses, visna, maedi, and ovine progressive pneumonia viruses (Sigurdsson et al., 1957; 1960; Sigurdsson and Pálsson, 1958; Kennedy et al., 1968). Various nomenclature has been used previously to describe the bovine lentivirus (Van Der Maaten et al., 1972; Boothe and Van Der Maaten, 1974; Georgiades et al., 1978). The current designation, BIV, is more contemporary and was adopted after the bovine lentivirus was characterized in greater detail by Gonda and colleagues (1987) who found that structurally, immunologically, and genetically, it more closely resembled the human and non-human primate immunodeficiency viruses.

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Table 1
Genera of bovine retroviruses

Genus	Virus	Disease
Lentivirinae	Bovine immunodeficiency virus (BIV)	lymphadenopathy lymphocytosis encephalitis
Spumavirinae	Bovine syncytial virus (BSV)	unknown
Oncovirinae	Bovine leukemia virus (BLV)	leukemia/lymphosarcoma
	Bovine Type D virus	unknown

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Studies that led to the isolation of the first bovine lentivirus, BIV, can be traced to field work done on a herd of dairy cattle at the Southeast Louisiana Experiment Station at Franklinton, Louisiana, by Dr. Cameron Seger, a veterinarian at the Louisiana State University Agricultural Center, and by his collaborator, Dr. Martin Van Der Maaten from the National Animal Disease Center, who isolated and initially characterized the first bovine lentivirus (Van Der Maaten et al., 1972). The Louisiana dairy cows studied by Dr. Seger had increased circulating white cell counts. The elevated leukocyte counts were attributed to an increase in the number of blood lymphocytes, a condition referred to as lymphocytosis. Persistent lymphocytosis is frequently associated with the development of enzootic bovine leukemia/lymphosarcoma (Burny et al., 1980); however, the etiologic agent of this disease, BLV, had not been clearly identified at the time and it was hoped that these studies would produce a candidate viral agent.

Dr. Seger focused his attention on one pregnant dairy cow (R-29), an 8-year-old Holstein, whose white cell counts were elevated and whose physical condition steadily worsened. After delivering her calf, she became severely emaciated and weakened, and all attempts to reverse this condition failed. It was speculated that she had lymphosarcoma because of the high white cell counts. After her death, necropsy was performed; enlarged nodes were found but the usual tumors associated with leukemia/lymphosarcoma were absent. Tissues were therefore sent to Dr. Van Der Maaten for further evaluation and determination of the possible cause of the disease (Van Der Maaten et al., 1972). Histological examination of these tissues revealed a generalized follicular hyperplasia of lymph nodes and perivascular cuffing in the brain. A syncytia-inducing virus with the morphology of a lentivirus was rescued from the tissues of cow R-29, and additional isolates were subsequently obtained from two other animals in the herd. Experimental inoculation of the tissue culture-rescued virus from cow R-29 into colostrum-deprived, isolation-reared, young calves induced a mild lymphocytosis and lymphadenopathy that could readily be detected in the subcutaneous lymph nodes. This condition persisted for several months. Histological examination of the lymph nodes of the experimentally infected calves revealed a follicular hyperplasia as observed in cow R-29. Lentivirus could be rescued from leukocytes of experimentally infected animals over a period of several weeks to several months.

Since the anticipated prominent clinical syndrome of enzootic bovine leukemia/lymphosarcoma did not appear in the experimentally infected calves, the animal

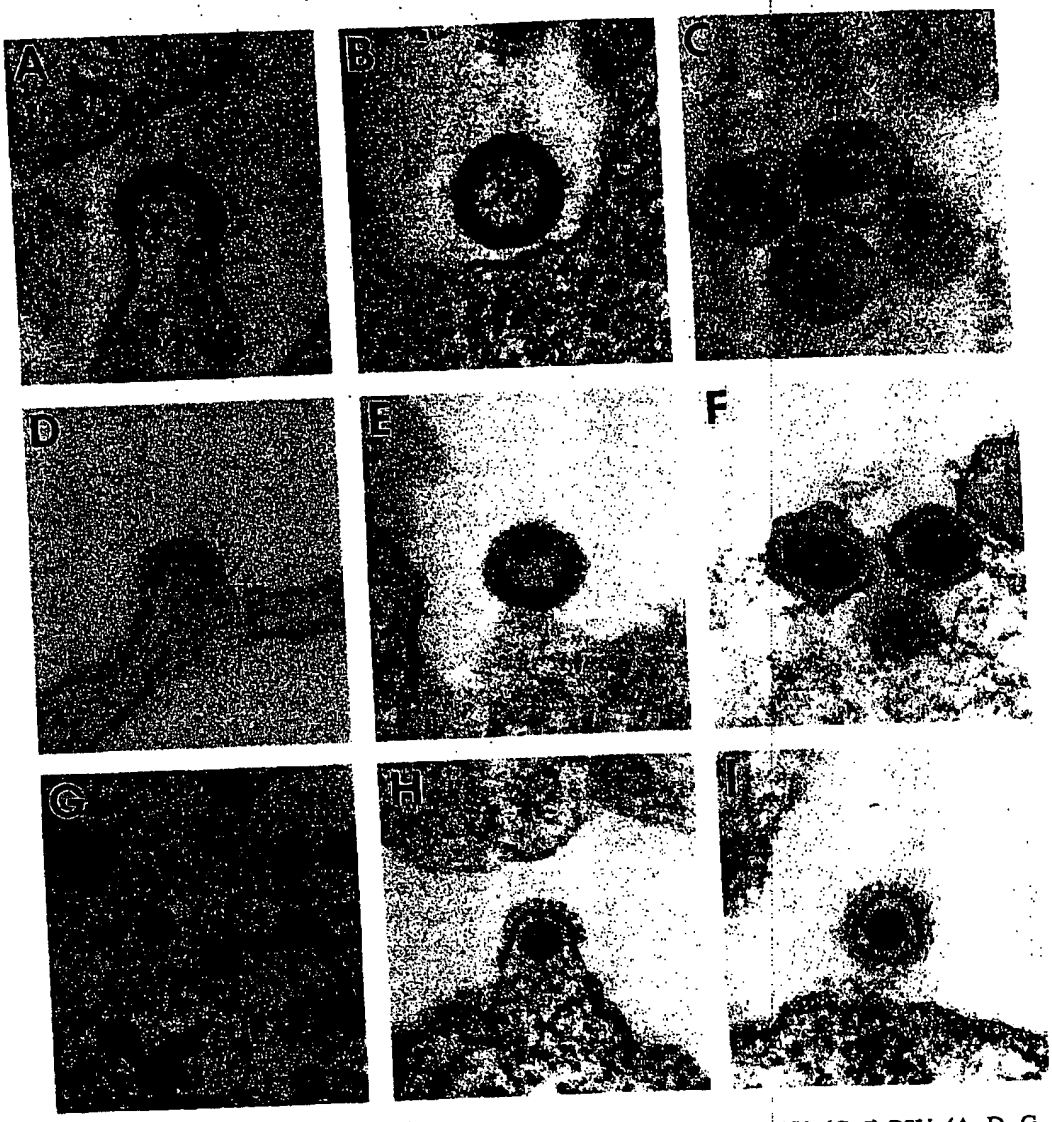

Fig. 1. Electron microscopy of BIV, BLV, and BSV. (A–C) BIV; (D–F) BLV; (G–I) BSV. (A, D, G, and H) Cell-associated or budding virus. (B and E) Immature extracellular virus. (C, F, and I) Mature extracellular virus.

study was terminated and the bovine lentivirus was put into low temperature storage. It was not until the human immunodeficiency virus type 1 (HIV-1), the cause of the acquired immune deficiency syndrome (AIDS), was found to be a lentivirus that there was a resurgence in interest in BIV and lentivirus research in general (Gonda et al., 1985; 1986; 1987). Initially, attention focused on the origins of the AIDS virus. Was HIV-1 derived from a lentivirus in farm animals that

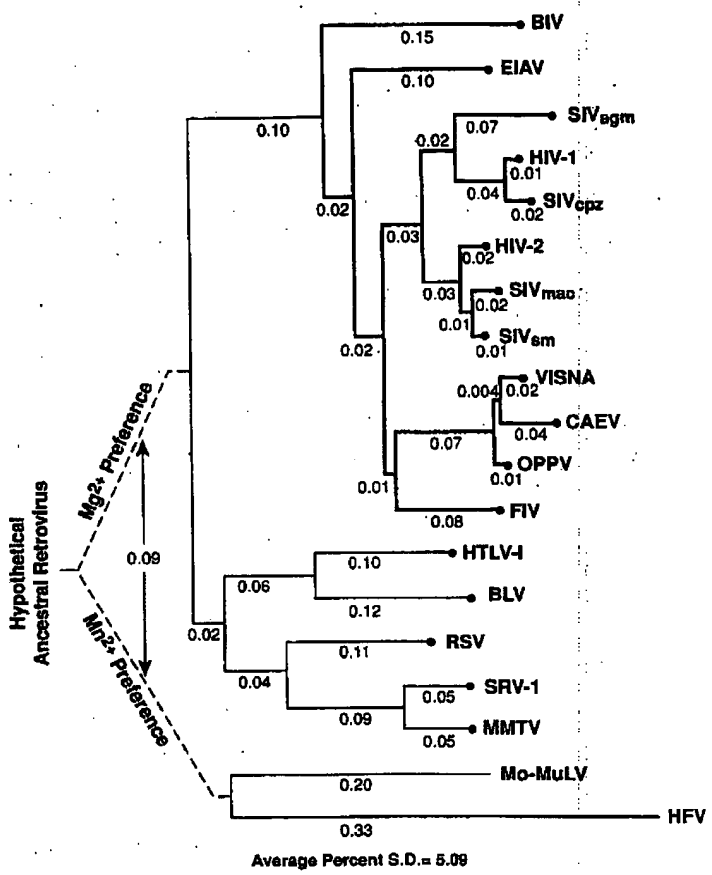

Fig. 2. Evolutionary relationship of BIV. Fitch-Margoliash phylogenetic tree of retroviral relationships based on the highly conserved 90 amino acids from the RT domain of the *pol* gene as described (Gonda et al., 1986; 1987; 1988; Garvey et al., 1990). Retroviruses used are visna virus, OPPV (ovine progressive pneumonia virus), CAEV (caprine arthritis encephalitis virus), SIVsm, SIVcpz, SIVmac, SIVagm (simian immunodeficiency viruses), HIV-2, HIV-1, EIAV (equine infectious anemia virus), BIV, FIV (feline immunodeficiency virus), HTLV-I (human T-cell lymphotropic virus type I), BLV, RSV (Rous sarcoma virus), SRV-1 (simian AIDS retrovirus), MMTV (mouse mammary tumor virus), Mo-MuLV (Moloney murine leukemia virus), and HFV (human foamy virus). Sequences were obtained from Garvey et al. (1990) and Genbank. Branch lengths are in units of $-\log M$, where M is the frequency of matching residues. The tree was rooted with HFV and Mo-MuLV as the outgroup taxons because they consistently had the lowest alignment scores and because their RTs preferentially use Mn^{2+} rather than Mg^{2+} as co-factor. The average standard deviation of the tree was 5.09 percent. (From Gonda, 1992, with permission of Current Science, Ltd., London.)

passed into man? Evolutionary analyses based on comparisons of conserved sequences in the reverse transcriptase (RT) domain of the *pol* genes of the lentiviruses and other retroviruses (Fig. 2) quickly dispelled this notion and demonstrated that: (1) the known lentiviruses are a genetically distinct group of retroviruses that cluster together; (2) HIV-1 (and HIV-2) are lentiviruses; (3) each lentivirus isolated from a different species is quite unique within the lentivirus

genus; and (4) BIV is relatively equidistant from all of the other lentiviruses for this conserved segment of DNA (Gonda et al., 1986; 1987; Garvey et al., 1990; Gonda, 1992). Subsequently, when an HIV-1 equivalent was not found, the interest in non-human lentiviruses shifted to finding ways to use them as models for HIV-1 infection and AIDS research.

2. Seroprevalence

Recent seroepidemiologic data on BIV prevalence suggest that viral infections are worldwide. Serologic screening of randomly selected cattle sera for BIV antibodies has shown a non-uniform distribution in the USA (Amborski et al., 1989; Black, 1990; Whetstone et al., 1990; Cockerell et al., 1992; Luther et al., unpublished data). Sera from eastern or northern parts of the USA rarely are positive for BIV (Gonda et al., unpublished data). In contrast, approximately 4% of randomly selected sera from southern or southwestern regions of the USA are BIV positive (Black, 1990). In the South, the average frequency within individual herds can be considerably higher. For example, average frequencies of 40% in beef and 64% in dairy herds were found in the Louisiana area; notably, many of these BIV-positive animals were also infected with BLV (Luther et al., unpublished data). Serological studies in other parts of the world have detected BIV in cattle in the Netherlands, Switzerland, Canada, Costa Rica, Venezuela, and New Zealand (Horner, 1991; Horzinek et al., 1991; Jacobs et al., 1992; H. Lutz, J. Bonilla, and R. Walder, personal communications).

All of the serologic studies performed to date have used the same isolate of BIV (R-29) as the antigen source (Amborski et al., 1989; Black, 1990; Whetstone et al., 1990; Horzinek et al., 1991; Cockerell et al., 1992; Horner, 1991; Luther et al., unpublished data). The exclusive use of BIV R-29 antigen in serological screens may make it difficult to distinguish between low-serum antibody titers to R-29-like BIV or new BIV variants. In support of this, emerging data suggest that a variant of BIV, which is serologically distinct from R-29, may exist in eastern USA cattle populations. The BIV-positive sera from eastern cattle produce only weak reactions in BIV ELISAs, radioimmunoprecipitate only the major BIV core protein, p26 (which contains the major Gag antigenic determinant conserved between lentiviruses) (Garvey et al., 1990; Battles et al., 1992), from isotopically labeled virions (Fig. 3), and are found in areas not predicted to have BIV infection (Gonda et al., unpublished data). A similarly weak reactivity pattern with BIV R-29 antigen has been observed in cattle in the Netherlands and Switzerland (Horzinek et al., 1991; H. Lutz, personal communication). These data suggest that several distinct strains of BIV may exist worldwide. Virus isolations and detailed comparisons are needed to confirm these observations.

3. Clinical and pathological features

A distinguishing feature of lentiviruses is their ability to induce a persistent lifelong infection in spite of a strong host immune response. There is usually a long

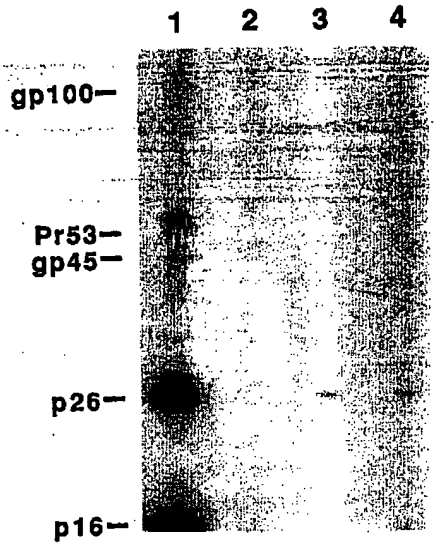

Fig. 3. Radioimmunoprecipitation of BIV-127 proteins by bovine sera from the southern and eastern USA. Lanes (1) BIV-infected cow serum from Louisiana; (2) uninfected negative control bovine serum; (3) and (4) cattle sera obtained from a single herd in the eastern USA. The location of gp100, Pr53, gp45, p26, and p16 immunoprecipitated by the various sera is shown to the left of the figure.

incubation period (months to years) before induction of disease. During the initial stages of infection, the host may appear clinically normal and only fine observation or serological assay can provide details of the infection process. Disease, when present, normally affects multiple organs and likely requires the presence of appropriate co-factors for full expression. Two primary sites of virus replication and pathogenesis are the immune system and brain. The clinical manifestations of lentivirus infections vary between each virus–host system as noted in Table 2.

The significance of natural BIV infections to the health of herds has not been clearly established. Two reasons for this are that cattle are production animals with a high turnover rate and that many cattle often become dually infected with BLV and BIV, making conclusions about the contribution of BIV to disease difficult. Nevertheless, in a long-term study (> 4 years) of a dairy herd in Louisiana with significant BIV and BLV infection (> 80%), notable observations were made (Luther et al., unpublished data). These animals had a high cull rate due to poor health, and many exhibited skin infections that were unresponsive to treatment. Histological examination of brain tissue from BIV-infected animals in this dairy herd revealed a non-suppurative perivascular cuffing indicative of a viral meningoencephalitis; these central nervous system lesions were similar to those described earlier by Van Der Maaten et al. (1972) and could not be attributed to infection by another virus.

Further information on the role of BIV in disease has come from observations on experimentally infected calves. Several studies that used the tissue culture-adapted BIV R-29 isolate have demonstrated a transient lymphocytosis and

lymphadenopathy as observed in cow R-29 without any overt clinical signs (Van Der Maaten et al., 1972; Carpenter et al., 1992; Onuma et al., 1992; Suarez et al., 1993). A long-term study (> 2 years) of cattle infected with molecularly cloned virus obtained from the BIV R-29 (Braun et al., 1988) and new BIV field isolates has shown that infected animals allowed to pasture freely without significant stress also developed a persistent lymphadenopathy but otherwise remained healthy for the duration of the experiment. At necropsy, enlarged lymph nodes were observed; histopathologic examination revealed a significant follicular hyperplasia of germinal centers (Oberste et al., in preparation) (Fig. 4). As observed in naturally infected animals, non-suppurative perivascular cuffing in the cerebrum, indicative of viral encephalitis, was present in brain tissue (Fig. 5). Carefully controlled long-term studies in cattle are needed to determine the significance of these findings to animal health. Animal studies in surrogate hosts described below provide additional experimental evidence as to the pathogenic potential of BIV gene expression in vivo.

Immune dysfunction, often associated with lentivirus infections, has been investigated in animals infected experimentally with BIV. Onuma et al. (1992) have shown that BIV infection of cattle reduces the responsiveness of various important monocyte functions without a change in CD4/CD8 ratios; there was also a slight delay in humoral antibody response to mouse antigen. Other studies examining the effect of BIV infection on immune function have demonstrated either mild or no immunosuppression on the basis of lymphocyte blastogenesis tests, neutrophil function tests, mononuclear subset analysis, and histopathological changes (Martin et al., 1991; Carpenter et al., 1992; Flaming et al., 1993).

Table 2
Clinical manifestations of lentivirus infections in natural hosts

Lentivirus	Disease description
Ovine visna, maedi, and progressive pneumonia virus	Progressive lethal pneumonia, chronic encephalomyelitis, spasticity, paralysis, lymphadenopathy, mastitis, generalized wasting, opportunistic infections
Caprine arthritis encephalitis virus	Generalized wasting, chronic leukoencephalomyelitis, progressive arthritis, osteoporosis, paralysis
Equine infectious anemia virus	Fever, persistent viremia, hemolytic anemia, lymphoproliferation, immune-complex glomerulonephritis, bone marrow depression, central nervous system lesions
Bovine immunodeficiency virus	Persistent lymphocytosis, lymphadenopathy, central nervous system lesions, weakness, emaciation
Feline immunodeficiency virus	Immunodeficiency-like syndrome, generalized lymphadenopathy, leukopenia, fever, anemia, emaciation, opportunistic infections
Simian immunodeficiency virus	Immunodeficiency, neuropathologic changes, wasting, opportunistic infections
Human immunodeficiency virus	Immunodeficiency, lymphadenopathy, opportunistic infections, encephalopathy, emaciation, Kaposi's sarcoma and other cancers

Adapted from Gonda, 1988, with permission of Wiley-Liss/John Wiley and Sons, Inc., New York.

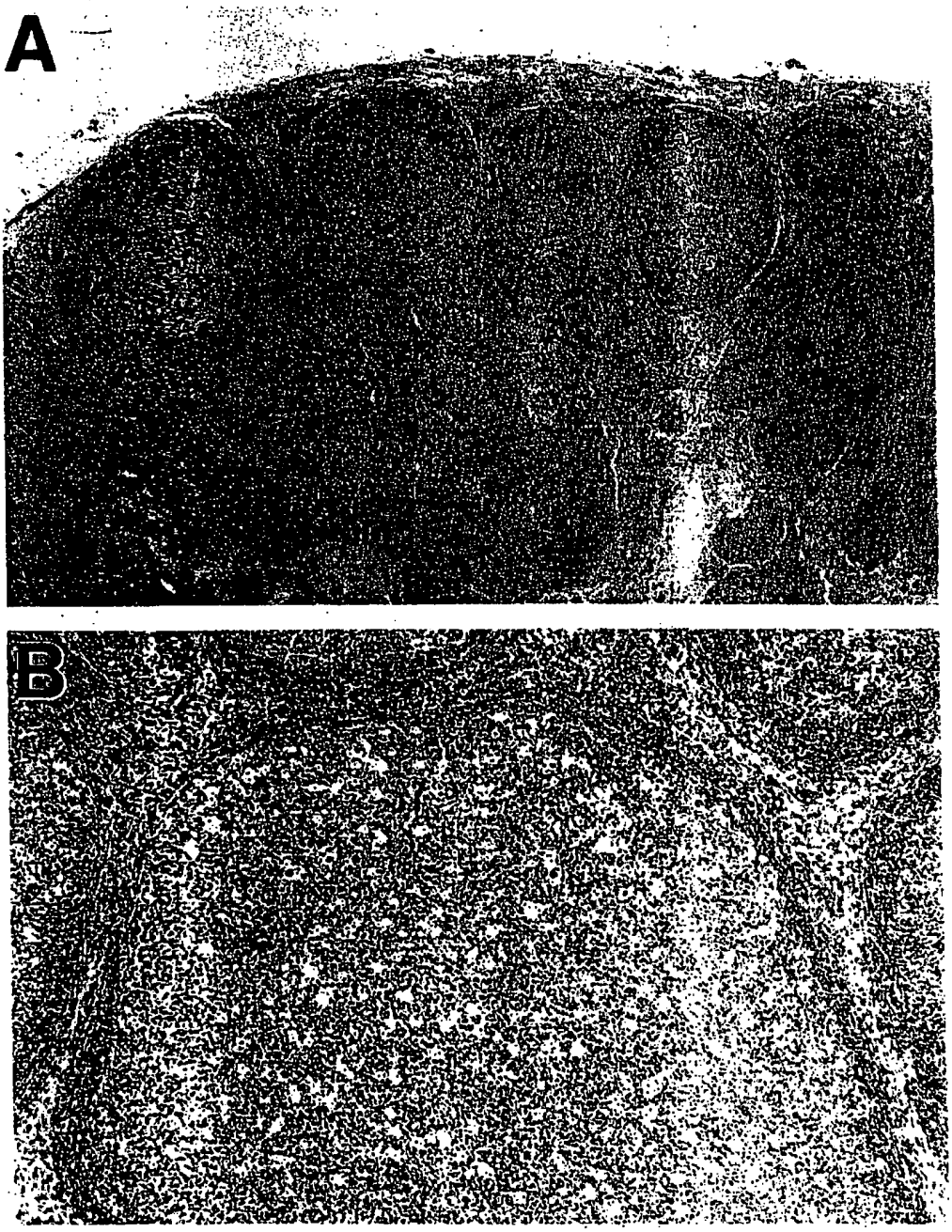

Fig. 4. Follicular hyperplasia of germinal centers of a subcutaneous lymph node from an experimentally infected cow. (A) Low magnification of subcapsular regions showing numerous enlarged germinal centers. ×350. (B) Higher magnification of germinal center in (A). ×1200.

4. Possible modes of transmission

BIV is transmitted experimentally by the intravenous inoculation of infected whole blood and cell-free and cell-associated virus. The most infectious inoculum is cell-associated material. Young calves (3 months of age) and older animals are susceptible to both natural and experimental infections with BIV. It is not known if BIV is transmitted in utero or whether newborns are susceptible to infection.

Although experiments to delineate the natural routes of infection for BIV have not been performed, observations on the modes of spread for other ungulate lentiviruses, evaluation of herd management practices, and data generated from seroprevalence studies of BIV and BLV allow for some speculation on the possible modes of transmission. The profound overlap of BIV and BLV infections in individual animals and herds in southern USA locales suggests that these two viruses may share common modes of transmission. In addition, BIV is more prevalent in dairy than in beef herds, suggesting that practices associated with dairy farming contribute to its spread. These may include the reuse of blood-contaminated items (e.g., needles used in the numerous vaccinations and bleedings and gloves worn during rectal palpations), the communal sharing of colostrum by newborns to enhance their immunity, and the failure to cleanse instruments used in invasive procedures such as dehorning. There is no evidence that casual contact

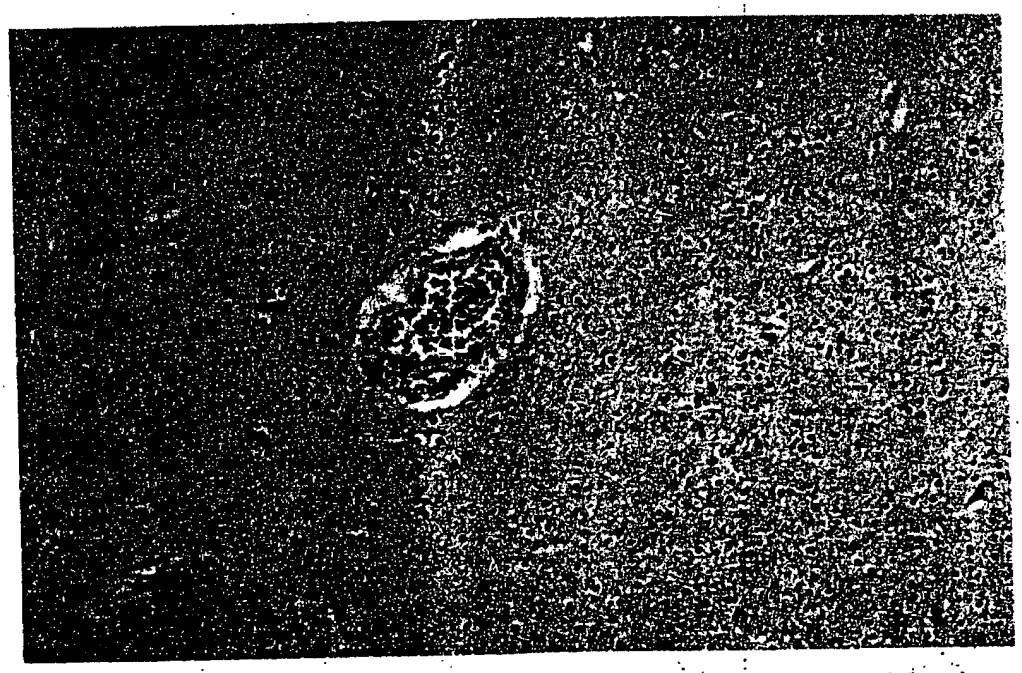

Fig. 5. Perivascular cuffing of mononuclear cells in the cerebrum of an experimentally infected cow. ×1200.

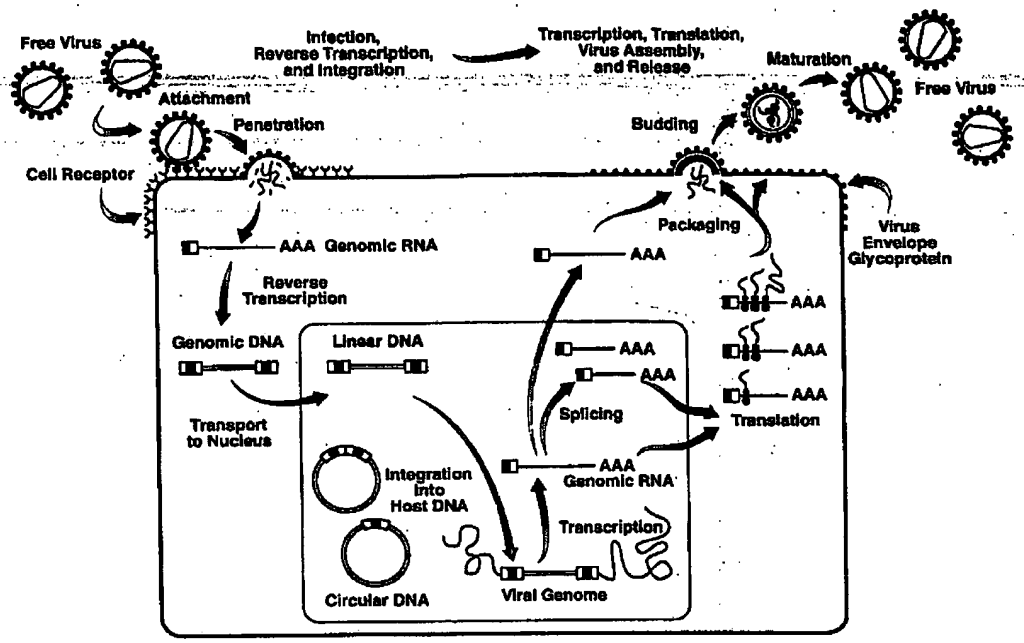

Fig. 6. BIV infection cycle. (From Gonda and Oberste, 1992, with permission of Marcel Dekker, Inc., New York.)

or insects play a role in the transmission of BIV, although it is known that equine infectious anemia virus and BLV can be transmitted by insects (Issel et al., 1990).

5. Molecular biology

BIV infection cycle

BIV is an exogenous retrovirus with a replication cycle similar to that of other members of the lentivirus genus (Fig. 6). Viral particles consist of two positive-sense, single-stranded, protein-encapsidated RNA genomes surrounded by an envelope containing viral and cell proteins. The envelope is derived from the plasma membrane of host cells during budding. The infection process initiates when free BIV particles attach to specific cell surface receptors via the envelope glycoproteins of the virus and penetrate the cell by direct fusion of the viral envelope with the plasma membrane. This event releases genomic RNA and mature *pol* gene products from the core of the virus into the cytoplasm, where the concerted action of the viral RT and ribonuclease converts the viral RNA into double-stranded DNA. The double-stranded viral DNA molecule, termed the provirus, is transported to the nucleus where integrase (IN) catalyzes its incorporation into the host genome. The integrated provirus can remain transcriptionally silent until appropriate cellular signals activate gene expression from the viral long terminal repeat

(LTR). Cell-mediated expression from the viral LTR is significantly enhanced by the action of the virally encoded Tat protein. Splicing of the primary genome-length viral mRNA into subgenomic messages and transport to the cytoplasm is carried out by the cellular splicing machinery and another virally encoded protein, Rev. Genomic and subgenomic viral messages are translated on ribosomes in the cytoplasm of the infected cell. Viral precursors for Gag and Gag-Pol assemble beneath the plasma membrane and incorporate viral genomic RNA during the process of budding. The viral envelope is decorated with surface (SU) and transmembrane (TM) glycoproteins. Following release, Gag-related precursors in the immature particle are cleaved into their functional subunits by the viral protease (PR) as the virus undergoes morphogenesis into a mature infectious particle. The mature particle can begin the infection cycle again by binding to a naive cell expressing the appropriate receptor for BIV.

Genome organization and transcripts of the BIV provirus

Molecular cloning and sequencing of proviruses from BIV-infected cells have been used to develop a complete genetic map of BIV (Braun et al., 1988; Garvey et al., 1990). The deduced genetic complexity of BIV has been reinforced by Northern blotting and cDNA cloning experiments used to characterize viral transcripts (Oberste et al., 1991; Liu et al., 1992; Oberste et al., 1993; Pallansch et al., submitted).

The BIV genome (Fig. 7) is 8,960 base pairs in the form of the proviral DNA. It contains the obligatory retroviral structural genes, *gag*, *pol*, and *env*, flanked on the 5′ and 3′ ends by the LTRs. BIV also contains the complex lentivirus 'central region' between and overlapping the *pol* and *env* reading frames.

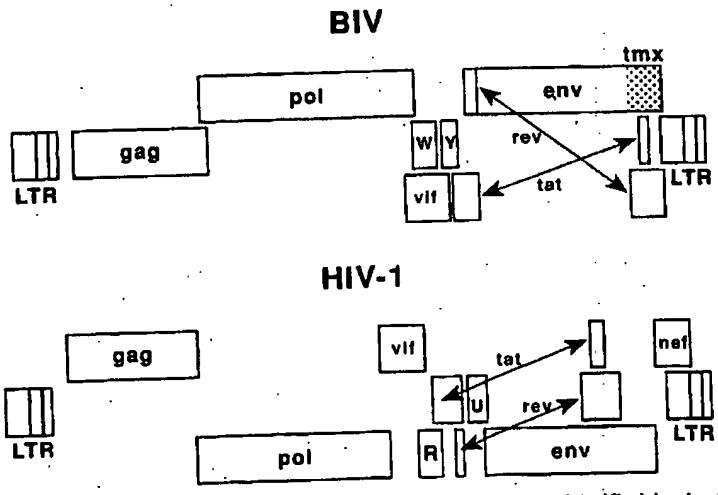

Fig. 7. Genome organization of BIV and HIV-1. The structural genes identified in the BIV and HIV-1 genomes are *gag*, *pol*, and *env*. The non-structural accessory genes identified in the BIV genome are *vif*, *tat*, *rev*, *vpy* (Y), *vpw* (W), and *tmx* and in HIV-1 are *vif*, *tat*, *rev*, *vpr* (R), *vpu* (U), and *nef*. (From Gonda et al., 1990b, with permission of the editors and Elsevier Science B.V., Amsterdam.)

The central regions of the lentiviruses contain coding exons for the non-structural accessory genes. Some of the non-structural accessory genes have been referred to as non-essential genes since their deletion or inactivation has little or no effect on virus replication in vitro, although profound effects have been noted for some in vivo (see for example Kestler et al., 1991). The non-structural accessory genes of HIV are the best characterized and include *vif* (viral infectivity factor), *tat* (*trans*-activator of transcription), *rev* (regulator of virus expression), *vpr* (another potential *trans*-activator), and *vpu* (virus release factor); there is an additional accessory gene, *nef* (negative factor), found at the 3′ end of the genome (Fig. 7) (Gonda and Oberste, 1992).

The central region of BIV contains coding exons of several putative non-structural accessory genes including *vif*, *tat*, *rev*, *vpw*, and *vpy*. Although BIV has no equivalent *nef* open reading frame as found in the primate lentiviruses, analysis of cDNAs of BIV transcripts have identified a unique gene, termed *tmx*, that consists of a single exon overlapping the 3′ end of the *env* gene and the U3 region of the LTR, which may be analogous to *nef* (Gonda, 1992; Greenwood et al., in preparation). Although there is limited sequence similarity, the genome locations, conserved traits in the predicted products, and/or functional studies of the BIV *tat*, *rev*, *vif*, *vpy*, and *vpw* genes suggest that they are probably analogous to the *tat*, *rev*, *vif*, *vpr*, *vpu*, and *vpx* genes of the primate lentiviruses (Garvey et al., 1990; Oberste et al., 1991; Liu et al., 1992; Oberste and Gonda, 1992; Pallansch et al., 1992; Carpenter et al., 1993; Oberste et al., 1993; Pallansch et al., submitted). BIV has at least nine putative genes and is the most complex non-primate lentivirus characterized to date.

As anticipated from the organization of the viral genome, the transcriptional pattern of BIV is also very intricate (Fig. 8). An analysis of expression and processing of the viral mRNAs has provided clues as to important regions in the genome that regulate BIV replication. Northern blot analysis has demonstrated that there are at least five size classes (8.5, 4.1, 3.8, 1.7, and 1.4 kb) of BIV-specific viral RNA in infected cells (Oberste et al., 1991). Complete cDNAs for the *tat*, *rev*, and *tmx* transcripts have been cloned and analyzed. The remaining gene transcripts (*gag* and *gag-pol*, *env*, *vif*, *vpw*, and *vpy*) have been deduced from Northern blot analysis or partial mapping of splice junctions defining their coding exons (Oberste et al., 1991; Liu et al., 1992; Oberste et al., 1993; Pallansch et al., submitted).

Viral structural proteins

Gag and Gag-Pol. The Gag and Gag-Pol products are derived from translation of the full-length viral transcript. The Gag precursor, Pr53, serves to initiate budding and incorporation of genomic RNA into the forming virus particle and is the minimal particle-forming unit of the virus (Rasmussen et al., 1990). The Gag products have been extensively characterized immunologically (Battles et al., 1992) and by amino acid analysis (Tobin et al., submitted). In the case of BIV, there are three major Gag products derived from proteolytic processing of Pr53. They are p16, p26, and p7, the matrix (MA), capsid (CA), and nucleocapsid (NC) proteins,

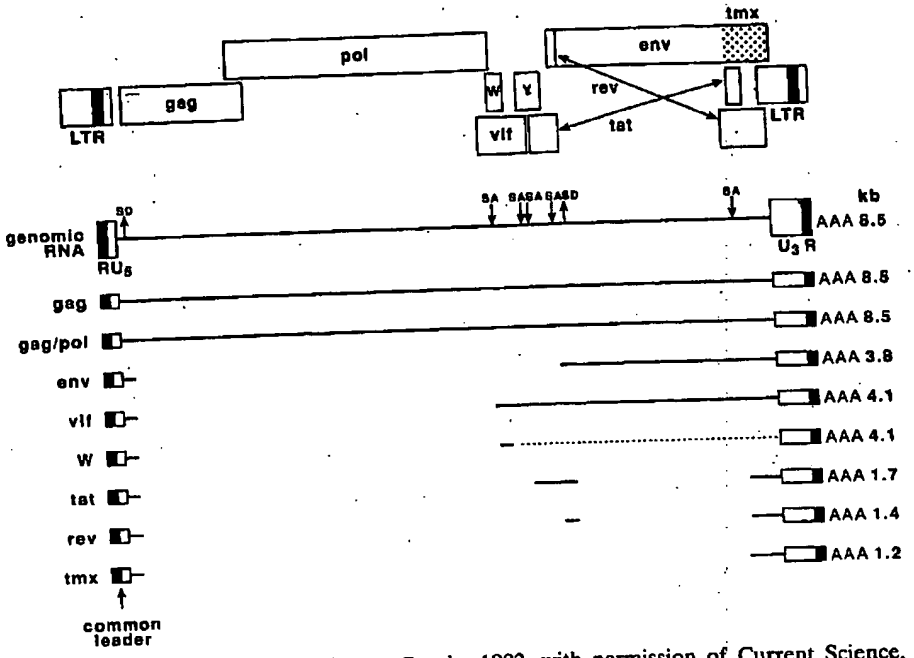

Fig. 8. Transcriptional map of BIV. (From Gonda, 1992, with permission of Current Science, Ltd., London.)

respectively (Fig. 9). The p7 protein migrates as a 13-kDa protein on gel electrophoresis; it is unresolved as to whether the apparent mobility of p13 is a result of dimerization or unusual charge properties of p7 (Battles et al., 1992; Tobin et al., submitted). The BIV CA and NC proteins are immunologically related to their homologues in HIV-1 (Gonda et al., 1987; Rasmussen et al., 1990; Battles et al., 1992; Jacobs et al., 1992; Whetstone et al., 1992). Interestingly, several small proteins (1.9 to 2.7 kDa) of unknown function are also present between the MA-CA and CA-NC cleavage sites and at the COOH terminus. The BIV Gag precursor is not modified with fatty acids as observed with most other retroviruses (Battles et al., 1992; Tobin et al., submitted). The Gag-Pol precursor is translated from the primary transcript by a −1 frameshift (Jacks et al., 1988; Varmus, 1988) and has been found to be a protein of 170 kDa in BIV (Rasmussen et al., 1990; Battles et al., 1992). The *pol* gene products, PR, RT, and IN, have not been immunologically identified with monospecific antisera. The locations of the Gag and Pol products in the virion are shown in Fig. 10. The BIV *pol* gene may encode a fourth product of 75 amino acid residues between RT and IN in the region assigned dUTPase activity in EIAV, FIV, CAEV, and ovine lentiviruses (Elder et al., 1992; Threadgill et al., 1993). The sequence in this region of *pol* in BIV (Garvey et al., 1990) is not related to proteins with dUTPase activity and thus is of unknown function.

Env. The SU and TM envelope proteins, gp100 and gp45, respectively, are heavily glycosylated proteins derived from translation of the *env* subgenomic

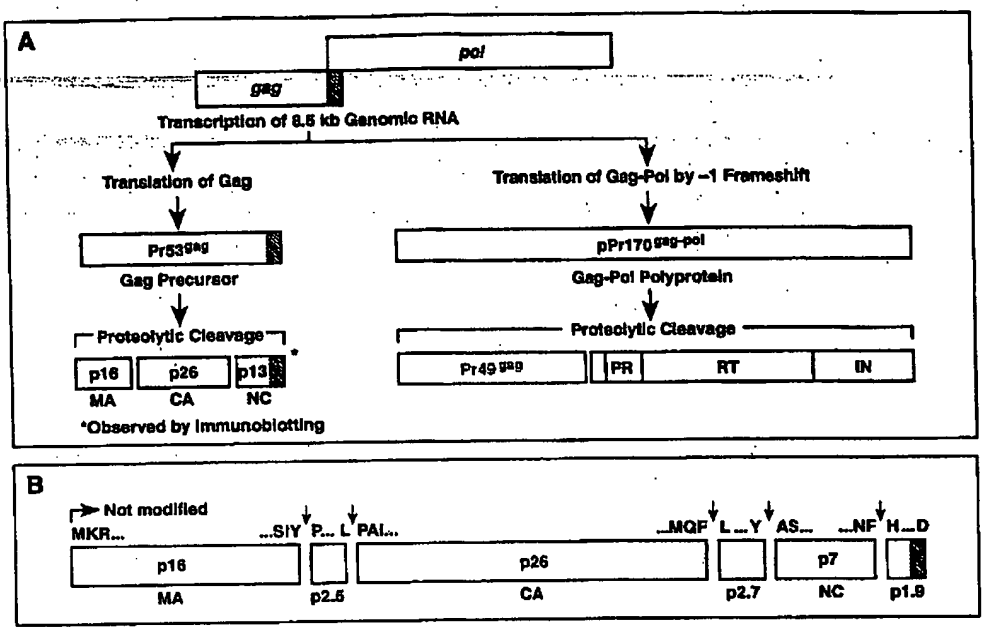

Fig. 9. Processing of the BIV Gag and Gag-Pol precursors. The processing of the BIV Gag precursor by the viral PR was determined experimentally by immunologic methods (A) and amino acid sequence analysis (B). The processing of the BIV Gag-Pol precursor (A) is that predicted from analyses performed with other lentiviruses.

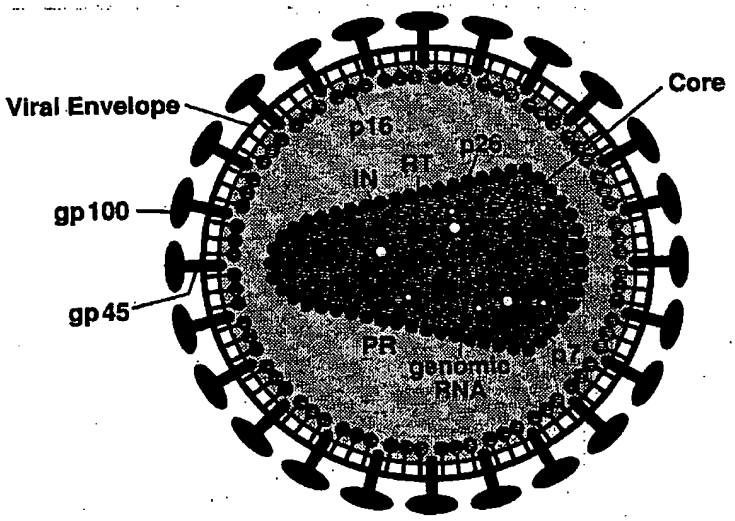

Fig. 10. Schematic representation of the mature BIV virion and location of the structural gene products. Proteins that make up the viral envelope are gp100, gp45, and p16. Proteins that are found in the viral core are p24 (CA), p7 (NC), PR, RT, and IN. The genome length viral nucleic acid is also found in the viral core.

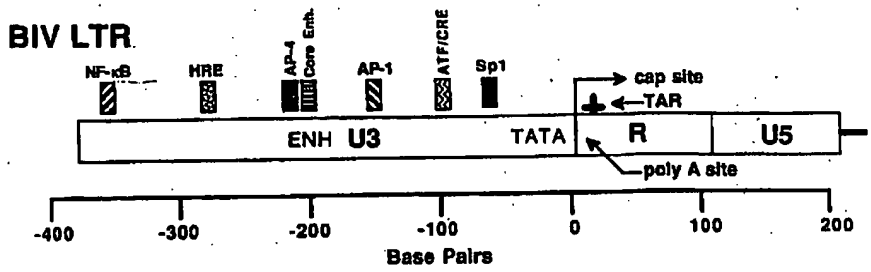

Fig. 11. Structure of the BIV LTR.

message and subsequent proteolytic processing (Rasmussen et al., 1992) (Fig. 10). By analogy with other lentiviruses, the SU envelope glycoprotein is the viral receptor, which resides entirely on the outside of the virus particle, and the TM protein is the primary fusigenic protein, which transverses the viral envelope and anchors the SU envelope protein to the virus particle. The TM envelope glycoprotein is believed to be responsible for the syncytia-inducing capacity of this virus.

Viral LTR and non-structural accessory proteins

LTR. Regulation of BIV replication is dependent both on viral and cellular *trans*-acting factors that interact with the viral LTR (Liu et al., 1992; Pallansch et al., 1992; Carpenter et al., 1993; Pallansch et al., submitted). The organization of the BIV LTR is consistent with that of other retroviruses in that it contains U3, R, and U5 elements (Fig. 11). The viral LTR is similar to eukaryotic promoters in being able to regulate gene expression, and in fact, contains transcription factor binding sites (e.g., NF-κB, Sp1, AP-1, and AP-4) that are present in some cellular promoters. The U3 region of the LTR contains the initiation, enhancement, and termination signals for transcription. Transcription initiation begins at + 1 in the BIV sequence, which marks the U3-R junction (Garvey et al., 1990). The R region of the BIV LTR, which is present in all viral transcripts, is believed to contain a putative *trans*-activating region (TAR) that interacts with the viral Tat protein to enhance viral gene expression as in HIV-1 (Carpenter et al., 1993; Pallansch et al., submitted).

Interestingly, the U3 region of the BIV LTR contains a single copy of several putative transcription factor binding sequences and at least one retroviral core enhancer (Fig. 11). This contrasts with the arrangement in more pathogenic lentiviruses with relatively high replication rates (e.g., HIV-1 and visna virus), which have two or more of these sites (Hess et al., 1989; Tong-Starksen and Peterlin, 1990; Ross et al., 1991; Andrésson et al., 1993; Chang et al., 1993; Gonda, in press). For example, BIV has single Sp1 and NF-κB sites, while HIV-1 has three Sp1 and two NF-κB sites; in addition, the BIV NF-κB site is relatively distal from the TATA box. These observations suggest that the number and placement of transcription factor binding sequences strongly influence the rate of viral replication and, as a consequence, pathogenicity. Study of these sites in the BIV LTR by

deletion analysis using LTR-reporter gene constructs has shown that several are important in upregulating virus transcription and that one, the core enhancer region, is absolutely essential for promoter function. Moreover, the relative contribution of each particular transcription factor binding sequence in upregulating gene expression is dependent on the species and tissue origin of the cell line used (Pallansch et al., submitted). Thus, the LTR appears to play a significant role in tissue-specific expression of the virus. Transcription factor binding sequences may have evolved within the LTR to enable the temporal and differential expression of the virus in target tissues.

Tat. The Tat protein (p14) of BIV is encoded by a multiply spliced message of three exons (Fig. 8) and is expressed early in the infection cycle. Two forms of *tat* cDNA have been found. One uses only the first coding exon (exon 2) and is translated into a Tat of 103 amino acids; the second uses the first 98 amino acids from the first coding exon (exon 2) and 10 amino acids from the second coding exon (exon 3) and makes a Tat of 108 amino acids (Pallansch et al., submitted). The presence of a two exon form of Tat parallels the organization observed for HIV-1 (Gonda and Oberste, 1992). *Trans*-activating function of Tat resides in exon 2 of the *tat* gene (Liu et al., 1992; Pallansch et al., submitted). BIV Tat is a phosphoprotein that localizes to the nucleus and nucleoli of infected cells. It is a potent *trans*-activator that enhances virus expression as much as 50- to 100-fold over that produced by endogenous cellular transcription factors alone in some cell lines. The mechanism of Tat activation has not been elucidated, but the protein appears to exert its effect by binding to the TAR RNA sequences present in the 5′ termini of all viral transcripts. The binding of Tat to TAR may localize it to the RNA polymerase II transcription complex in a manner that enhances viral gene expression (Rosen and Pavlakis, 1990). The putative TAR sequences of BIV have been localized by deletion analysis to between + 1 and + 20 nucleotides in the viral LTR; removal of these sequences completely abolishes Tat activity (Garvey et al., 1990; Carpenter et al., 1993; Pallansch et al., submitted).

It is noteworthy that the Tat proteins of BIV and HIV-1 are capable of *trans*-activating each other's LTRs, although the level of *trans*-activation is significantly less than in the homologous system (Liu et al., 1992; Pallansch et al., submitted). The locations of the BIV and HIV-1 TARs are similar in their respective LTRs. Although the TAR sequences in BIV are predicted to form a stem-loop structure, as does those of HIV-1, there is no obvious similarity in primary sequence (Garvey et al., 1990; Carpenter et al., 1993; Pallansch et al., submitted). It is not known if the BIV and HIV-1 Tat proteins *trans*-activate each other through an interaction with the heterologous TAR sequences or indirectly activate virus gene expression by activating specific cellular transcription factors whose binding sites are present in the LTRs.

Rev. The Rev protein (p23) of BIV is a phosphoprotein that localizes to the nucleus and the nucleoli of infected cells similar to Tat (Oberste et al., 1993). The BIV Rev protein is encoded by a multiply spliced, early expressed message of three exons. The second and third exons encode Rev, and the first coding exon (exon 2) of these two is derived from sequences at the 5′ end of and in frame with

translation of the *env* gene (Fig. 8). Complementation studies using *rev*-deficient BIV proviruses and *rev*-expressing cell lines have shown that Rev positively regulates the expression of unspliced and singly spliced viral transcripts, presumably through the action of a Rev-responsive element (RRE) (Oberste et al., 1993). Based on the splicing pattern of BIV transcripts, the BIV RRE is predicted to be located in the 3′ coding sequences in the region of *env* encoding the extracellular domain of the TM protein.

Tmx, Vif, Vpw, and Vpy. Tmx (p19) is encoded by a singly spliced message (Fig. 8). The Tmx p19 protein is found in the cytoplasm of infected cells and in the virion, and the *tmx* message has been found in infected animals. The *tmx* cDNA clones begin translation 14 nucleotides from the splice junction of the common leader and the second exon at the first AUG that is in frame with the *env* gene transmembrane coding sequences (Gonda, 1992; Greenwood et al., in preparation). Although not in a separate reading frame like HIV-1 *nef*, its location in the BIV genome is similar to that of the primate *nef* gene (Fig. 7). It is interesting to speculate that Tmx has a function similar to Nef. Biological studies to define the role of Tmx are currently in progress.

Little is known of the other accessory genes of BIV. BIV *vif* is found in the center of the genome overlapping but in a different reading frame than *pol* (Fig. 7) and is predicted to encode a protein of 23 kDa. Although there is little sequence similarity between the predicted Vif proteins of lentiviruses, there are two well-preserved sequence motifs that suggest a conservation of function (Oberste and Gonda, 1992). The splice junction for a cDNA encoding Vpw has been found (Fig. 8); Vpw is predicted to encode a protein of 7 kDa. No discrete cDNA has been found for Vpy but it is predicted to encode a protein of 10 kDa (Gonda, 1992).

6. Host: virus interaction

Tropism

Lentiviruses infect cells of the immune system, primarily monocytes/macrophages and lymphocytes in vivo. The non-primate lentiviruses are also capable of infecting adherent cells in vitro (Gonda et al., 1987; Andrésson et al., 1993). The in vitro tropism of BIV is quite broad. BIV replicates in fibroblast-like cells and is cytopathic in most, causing syncytia and cell death (Fig. 12). Productive infections have been established in primary cultures of embryonic bovine spleen, brain, lung, choroid plexus, testes, thymus, kidney, and synovial membrane (Gonda et al., 1987). In addition, established canine thymus (Cf2Th), embryonic rabbit epithelium (EREp), and various other bovine cell lines have been infected (Gonda et al., 1990a; 1990b; Gonda, 1992). Only the Cf2Th cell line is known to sustain a long-term productive infection (Bouillant et al., 1989; Gonda et al., 1990a; 1990b). BIV has also been found to replicate in bovine leukocyte adherent cell lines with a macrophage-like morphology (Pifat et al., 1992) and bovine peripheral blood mononuclear cells (PBMC) (Onuma et al., 1992; Gonda et al., unpublished data). Although the in vitro tissue tropism of BIV is rather broad, the in vivo target cells

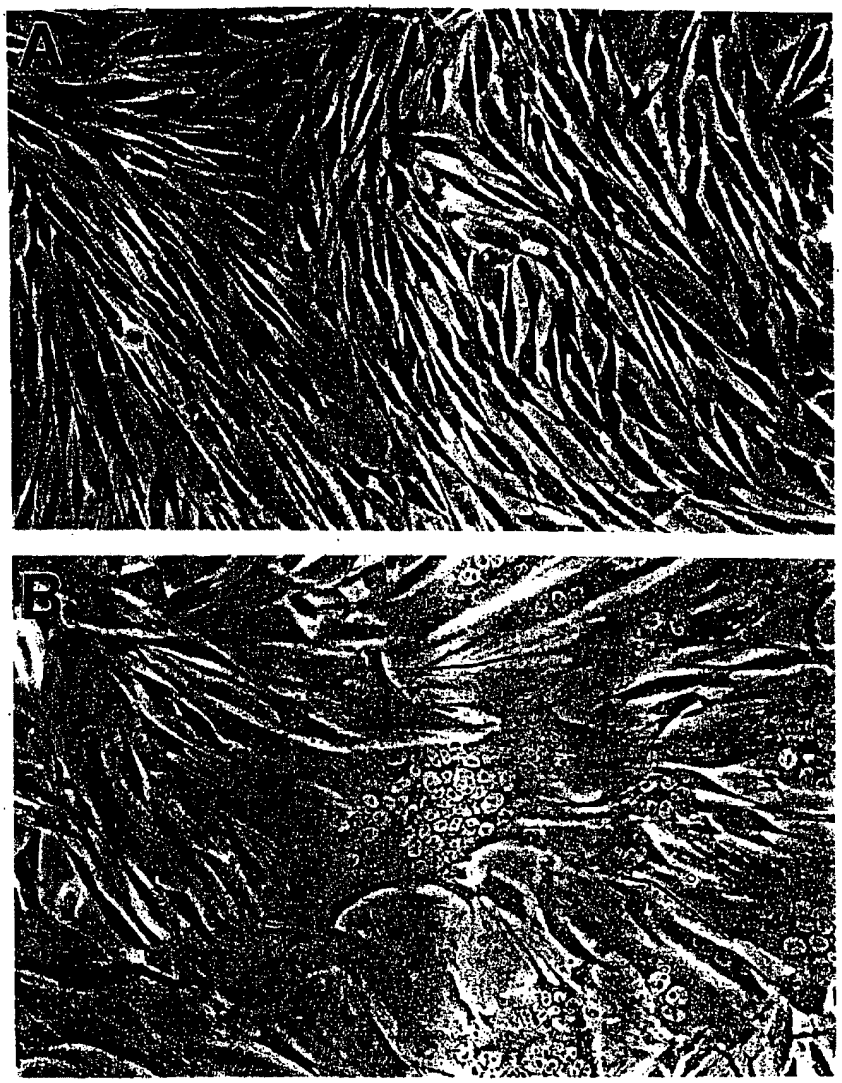

Fig. 12. Induction of syncytia in BIV-infected EREp cells. (A) Uninfected EREp cells. (B) BIV-infected EREp cells.

of BIV infection are immune cells and are most likely of monocyte/macrophage lineage (Carpenter et al., 1992; Onuma et al., 1992; Pifat et al., 1992). As evidence, there is a preferential isolation of BIV from peripheral blood monocyte- but not T-lymphocyte-enriched cultures, and monocyte-enriched cultures can be infected in vitro (Onuma et al., 1992). In infected animals, BIV DNA from spleen, lymph nodes, PBMC, lungs, and brain can be specifically amplified by polymerase chain reaction (PCR) (Pifat et al., 1992; Oberste et al., unpublished data); BIV RNA can be detected in PBMCs by in situ hybridization (Carpenter et al., 1992); and BIV

protein can be localized by in situ immunohistochemistry in atypical blastic splenic macrophages (Pifat et al., 1992). A clearer picture of the cell tropism will emerge when the cellular receptor has been cloned and characterized.

Interaction with surrogate hosts

Rabbits. Animal models may provide insight into disease mechanisms and virus–host interactions. The growth of BIV in rabbit cell cultures was indicative of its ability to induce persistent infections in specific pathogen-free New Zealand white rabbits (Pifat et al., 1992; Van Der Maaten and Whetstone, 1992). Rabbits can be infected with a single inoculation of cell-associated or cell-free virus, although the former is more efficient. The animals respond to the virus by mounting a rapid (within weeks), high-titered (ELISA titers $> 1:25{,}000$), and persistent (for the life of the animal) humoral immune response. A mild lymphadenopathy is seen early in the infection but there are no apparent clinical signs of disease. Virus can be rescued from PBMC, spleen, and lymph nodes, and these and other immune tissues and brain are the easiest to confirm as sites of BIV replication by PCR amplification of BIV sequences. In addition, in situ immunohistochemical staining of splenic macrophages with BIV-specific serum suggests that macrophages are the active sites of virus replication in rabbits (Pifat et al., 1992).

Rabbits can also be infected by BLV, although this is achieved with greater difficulty than with BIV (Wyatt et al., 1989). BIV and BLV were used in an ongoing cofactor study (> 2 years) to determine the potential for BIV to perturb the immune system and facilitate infection of rabbits by another virus (Gonda et al., unpublished data). This study demonstrated that BIV infection, which preceded or shortly followed inoculation with BLV, facilitates replication of BLV in rabbits. Rabbits in this study given BLV alone have not seroconverted to BLV suggesting that they did not become infected. The immune dysfunction described for the monocytes in BIV-infected cattle and the apparent replication of BIV in rabbit macrophages suggest that BIV interferes with the rabbits' ability to mount a cellular immune response to BLV, perhaps through dysfunction of monocytes and macrophages. The possible dysfunction of monocytes and macrophages in BIV-infected rabbits needs to be studied further.

Transgenic animals. Transgenic mice have played an important role in understanding the pathogenesis of gene expression for HIV-1 (Leonard et al., 1988; Kopp et al., 1993). Transgenic animals with a complete functional BIV provirus have been made (Pallansch et al., in preparation). Preliminary data on the biological effects of virus gene expression have been obtained with two lines of BIV provirus transgenic mice. Animals from both lines develop antibodies to BIV proteins and exhibit significant clinical disease including a neurologic syndrome. Pathology was observed in several organs including brain, thymus, spleen, lymph nodes, and skin, and one line exhibits early mortality (50%) attributable to a virally induced meningoencephalitis. Virus gene expression in these animals was low, could only be detected by PCR, was strongest in brain tissue, and generally correlated with pathology observed in the affected organs.

Other experimental inoculations. Inoculation of rodents (mice, rats, and guinea pigs) with BIV has been attempted; there is no evidence that these animals can sustain active virus replication (Gonda, 1992). Sheep and goats also have been inoculated with BIV-infected cells (Whetstone et al., 1991). In both species, antibodies to BIV p26 were detected early (2 weeks postinoculation) while antibodies to envelope and other putative viral peptides persisted for one year. Although the persistence of antibodies to BIV was used as presumptive evidence that the animals were infected, virus rescues were unsuccessful and detection of viral DNA was not attempted. Thus, it is not clear whether these animals were infected productively or merely received an immunizing dose of viral antigen.

7. Conclusions

The lentiviruses are a morphologically and genetically distinct group of exogenous retroviruses associated with inflammatory diseases and immunodeficiencies. Sigurdsson (1954) was the first to coin the term 'slow virus' in his description of disease induction by visna virus. It is appropriate that the name 'lenti' (latin for slow) was recommended as the nomenclature to describe this genus of retroviruses. Lentivirus disease is slow in onset, often involves multiple organs, and is severely debilitating, although not always fatal. As examples, HIV-1 in man and some strains of simian immunodeficiency viruses in monkeys induce a fatal immunodeficiency syndrome and encephalopathy. In contrast, caprine arthritis encephalitis virus in goats induces a chronic arthritis, encephalitis, and mastitis without significant mortality, and many of the simian immunodeficiency viruses in their normal hosts have no overt pathogenicity (Clements and Payne, 1994; Hirsch and Johnson, 1994; Sellon et al., 1994). The mechanism(s) by which each of the pathogenic lentiviruses induces disease is not clearly understood but certainly is related to the interaction of virus with host immune cells.

Monocyte/macrophages, which play a pivotal role in the immunobiology of the host (Varesio et al., 1990; Wiltrout and Varesio, 1990), are major in vivo target cells for infection by most lentiviruses (Narayan et al., 1982; Gendelman et al., 1989; Gartner and Popovic, 1990; Meltzer et al., 1990; Onuma et al., 1992). Primate lentiviruses also infect CD4 + lymphocytes (Dalgleish et al., 1984; Klatzmann et al., 1984). The direct cell killing of CD4 + lymphocytes by HIV-1 may be responsible for the depletion of helper T-cells and the resultant immunodeficiency that occurs in AIDS. Other mechanisms have also been implicated, including apoptosis, anergy, defective signaling, molecular mimicry, super-antigen-induced cell proliferation and depletion, and autoimmunity (Laurent-Crawford et al., 1991; Terai et al., 1991; Gougeon et al., 1993). In addition, aberrant induction of inflammatory cytokines appears to play a significant role in HIV-1 pathogenesis and may be a factor in AIDS encephalopathy (Rosenberg and Fauci, 1990; Vazeux et al., 1990; Matsuyama et al., 1991; Roederer et al., 1992; Staal et al., 1992).

The early pathogenesis of BIV in experimentally infected cattle is similar to that seen in early asymptomatic HIV-1 infection in humans (Carpenter et al., 1992;

Onuma et al., 1992; Suarez et al., 1993; Oberste et al., in preparation). Like HIV-1 and AIDS, several years may be required before significant BIV pathogenesis becomes readily apparent. Possibly, co-factors absent in controlled animal experiments, but present in natural infections, are needed to see the full pathogenic effects of BIV, as observed in feline immunodeficiency virus-infected cats (Pedersen et al., 1990).

Persistent lymphadenopathy and central nervous system lesions are common features of lentivirus infections (Table 2). In BIV-infected cattle, the brain lesions are suggestive of a viral encephalitis (Oberste et al., in preparation). Similar lesions are also found in some strains of BIV provirus transgenic mice (Pallansch et al., in preparation). BIV DNA has been demonstrated in lymph nodes and brains of infected cattle by PCR, and the expression of BIV in the brains of transgenic mice correlates with pathogenesis. The role of cytokines in central nervous system lesions has not been determined.

The lentiviruses contain complex genomes and regulatory mechanisms to insure their appropriate expression. BIV is similar in this regard and is the most complex of the non-primate lentiviruses (Kawakami et al., 1987; Garvey et al., 1990; Saltarelli et al., 1990; Gonda, 1992; Gonda and Oberste, 1992; Andrésson et al., 1993; Elder et al., 1993; Gonda, in press). In the acute stages of HIV-1 infection, there is a burst of virus replication which creates a significantly large pool of covertly infected immune cells which accumulate in lymphoid tissue (Embretson et al., 1993; Pantaleo et al., 1993). This burst is followed by a period of restricted viral replication called the asymptomatic phase. Originally, it was believed that lentiviruses would enter a latent phase in the host during this stage. It is now known that while a lentivirus may remain transcriptionally silent in an infected cell until appropriate endogenous or exogenous factors activate the LTR, the infection in the animal is never truly latent, as active replication is probably occurring somewhere in the body (Embretson et al., 1993; Pantaleo et al., 1993). Eventually, the animal succumbs to the accumulated effects of low-level virus replication, and overt disease results. In cattle experimentally infected with BIV, a similar pattern of virus replication occurs. Early in the infection (< 6 months), viral DNA can be detected by PCR in and virus can be rescued from PBMCs routinely. This becomes more difficult in later stages (0.5–2 years) of infection; nevertheless, viral DNA can be detected readily in immune organs by PCR (Oberste et al., in preparation). These observations indicate that immune organs are major repositories for virus replication.

One feature of lentivirus biology is genome variability (Hahn et al., 1986; Starcich et al., 1986; Payne et al., 1987; Saag et al., 1988; Gonda et al., 1989). Several proviral molecular clones from a single isolate of BIV have been sequenced in their entirety; these studies have shown that a significant amount of genomic variability exists within a single isolate, with the greatest variability occurring in the *env* gene (Braun et al., 1988; Garvey et al., 1990; Garvey et al., unpublished data). Recent sequence studies on new isolates of BIV have demonstrated that significant genetic variation exists between different strains (Suarez et

al., 1993; Oberste et al., in preparation). Variability of the viral genome enables it to evade immune selection pressures and to adapt to new niches in the host.

While the SIV infection in macaques appears to be the 'gold standard' in terms of an animal model for AIDS (Desrosiers and Ringler, 1989), no single virus-animal model will be sufficient for all aspects of HIV-1 and AIDS research (reviewed in Esparza and Osmanov, 1989; Gonda and Weislow, 1992). The replication of BIV in immune cells, its inability to infect humans or human cells, its induction of chronic infections in spite of a strong host immune response in small laboratory animals, and its shared antigenic, genetic, and pathologic features, all suggest that BIV infections could provide a useful model for testing antiviral strategies, if targeted to parts of the virus infection cycle most similar to those in HIV-1. Moreover, the comparative study of lentivirus biology in natural and surrogate hosts will provide a better understanding of virus–host interactions that are required for pathogenesis.

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